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A DISSERTATION SUBMITTED TO THE FACULTY OF THE DIVISION OF THE BIOLOGICAL SCIENCES IN CANDIDACY FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF BOTANY
1933

By
CLYDE HOMAN

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Reprinted from
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EFFECTS OF IONIZED AIR AND OZONE ON PLANTS

CLYDE HOMAN

(WITH FIVE FIGURES)

Introduction

Beginning as far back as the latter part of the eighteenth century, numerous investigators have subjected plants to the influence of electrical fields of widely different potentials and frequencies, using various methods of application, often with the idea of obtaining some growth-stimulating effect. Adequate controls were generally not maintained, nor were the conditions used possible of duplication owing to lack of measurements of environmental factors. Although results were reported varying from greatly increased yields to disastrous effects, later extensive and more carefully controlled experiments (**2, 6**) tend to disprove any effect on growth because of potential gradient alone.

About 1914 it was suggested that such effects on growth, as were observed when using electrical discharges, were not due to the effect of a potential difference on physiological functions of the plant, but merely to secondary factors resulting from the electrical discharge, *e.g.*, formation of ions, ozone, active oxides of nitrogen, etc., (**5, 10, 21**). As far as can be found from the literature, there have been no experiments in which the mentioned factors have been isolated. There has been no work done in which these factors have been measured by any reliable method, either when attempts were made to isolate them, or under the conditions of a high tension discharge.

Workers who have used ultraviolet rays, x-rays, and radioactive materials, as well as electrical discharges, have employed agents that ionize the air and produce ozone to a greater or lesser extent; and to them a knowledge of the effects of these factors is of interest.

Results of previous investigators

The first discussion of ionized air in this connection occurred in such papers as those of BRESLAUER (**1**) and JØRGENSEN (**10**). These papers refer to simple experiments with overhead wires, with the assumption that the air is more or less ionized by such discharges, but making no mention of measuring the ionization, if any, that may have occurred in the vicinity of the plants.

More recently experimental methods have been developed to determine whether ionized air has a direct influence upon plant growth. However, an analysis from the physicists' point of view of the previous experimental work with ionized air discloses a number of errors in method and interpretation sufficiently serious to invalidate most of the conclusions reached. Some of the most obvious of these errors may be pointed out.

Using polonium on copper foil as an ionizing agent for air, which was then passed over the plant material, MIDDLETON (**16**), WHIMSTER (**29**), VAN ASPEREN DE BOER (**28**) and PORTSMOUTH (**19**), obtained contradictory results. MIDDLETON found an increase in respiration varying from 5.58 ± 2.57 to 29.11 ± 5.62 per cent. PORTSMOUTH, attempting to duplicate MIDDLETON's results with barley seedlings, got no significant increase in one experiment, and only 1.5 per cent. in another. Again, WHIMSTER found an increase as high as 85.7 ± 7.1 per cent. in respiration of *Pelargonium* leaves, while PORTSMOUTH with a similar experimental setup obtained no sign of the increase found by WHIMSTER. Likewise VAN ASPEREN DE BOER found no effect of ionized air on the respiration of fungi.

These men assumed that only alpha particles are emitted by polonium. However, RUSSELL and CHADWICK (**20**) found both a gamma and a beta radiation, the gamma being three times as strong when the polonium was deposited on copper as when deposited on aluminum. Therefore the results of the experiments given above may have been influenced by these gamma and beta radiations.

In these experiments, moreover, the number of ions per cc. of air was calculated from the saturation current and the volume of air between two plates placed in the position occupied by the plant material used. Of course the field would extend beyond the plates, and ions would be drawn from a considerable distance; but it is of much greater importance that the use of such high potentials as 947 volts across plates 2–5 cm. apart resulted in the electric field simply drawing the ions to the plates as fast as they were formed (the polonium being at a distance of only 4–5 cm.). What they really calculated was the rate of production of ions. The *number of ions per cc.* would not be measured by this procedure. The amount of ionization at a given moment would depend partly on the rate of recombination of ions, which would greatly lessen the number, as would also diffusion of ions and the movement of air. The voltage used was so high that no other evidence is needed that the ions were drawn to the plates as fast as formed, the mobilities of the ions being about 90 cm. per second.

SPOEHR (**23**) drew air, supposedly ionized by sunlight, from outdoors over wheat seedlings and onions, and measured the CO_2 output during the day and night. He found that when the air was not de-ionized by passing through an electric field, the ratio of CO_2 output per hour by day to that by night was 1.15 for onions and 1.042 and 1.091 in two studies of wheat seedlings. In two experiments, in which he de-ionized the air drawn in before passing it over the plants, the ratios were 1.010 and 1.015. This would indicate that air exposed to daylight with its slightly higher degree of ionization caused slightly increased respiration, provided no other changes had also been brought about. It would seem, however, that drawing the air

through 45 feet of glass tubing in a thermostat would allow sufficient time for considerable recombination to take effect, possibly nullifying the higher ionization due to the sun's rays. There was no measurement of the degree of ionization of the air, reliance being put on the work of other investigators, which shows, in general, that there is a higher ionization of the air in sunlight than in darkness. There has evidently been no confirmation of this work.

HENRICI (**7, 8**) noted in the course of her experiments that the assimilation of alpine plants was much more active before a thunderstorm in spite of the low intensity of the light. She ionized the air artificially by the use of thorium oxide, and protected the plants by aluminum screens from any alpha radiation. She found that for lowland plants ionization favors assimilation in weak light, has no effect in medium light, and retards it in strong light; for alpine plants ionization increases assimilation in weak and medium light, and has no effect in strong light. She claims that in suitable conditions of light, ionized air increases photosynthesis; in some cases she got as high a ratio as 3–4:1. Her results, however, were not based on the averages of a sufficiently large number of experiments for calculating the probable error; hence it is not possible to estimate their value. Calcium hydroxide solution in Pettenkoffer tubes was used for absorption of CO_2 and it does not seem that the small differences in CO_2 found could have been very accurately measured. She has not continued with this work as far as can be determined from the literature, and there has been no confirmation from other sources. No measurements as to degree of ionization produced were made. Methods used to control temperature, etc., were not clearly described. Also, thorium oxide is a very weak ionizing agent, and aluminum sheets would not stop gamma rays.

LIPPERHEIDE (**14**), using sparks as a source of ionized air, presented the results and conclusions of a number of experiments mainly on the supposed effect of ionized air under the conditions in which he produced them. The conditions of his experiments are briefly as follows: Potted bean plants, *Phaseolus vulgaris,* were grown in garden soil in cases with glass tops. The condition of the air in the treated case was modified by blowing air through two metal combs placed opposite each other. Sparks were passed between the points by connection with an induction coil and other necessary electrical apparatus. Though he does not state the current or sparking distance used, the coil had a maximum capacity of 18 amp. and would give a spark 20 cm. long.

Only a relative idea of the degree of ionization in the treated air as compared to the control was obtained. This was determined by the time it took to discharge a metalfoil-leaf electrometer sufficiently to cause the leaf to move five divisions on the scale. Roughly, the degree of ionization would

be inversely proportional to the time elapsed. The peculiar thing about his figures is that they show great variability in the time required for this degree of discharge of the electrometer. In the control case the time varied from 1 minute and 40 seconds, to 4 minutes, most of the readings being about 4 minutes. In the treated air the variation was from 7 to 50 seconds, mostly around 10 seconds. Thus, the average degree of ionization of the treated air was roughly 24 times that of the control. This gives an ionization of only 24,000 per cc., if the ionization of the control air is assumed to be 1000 per cc. This is much lower than has been obtained by the writer and others referred to, and indicates that the sparks used were not of great number and the field strength comparatively weak. This probably accounts for his being able to grow plants under the conditions employed, for with a sufficiently weak field and sparks the quantity of ozone would be kept below toxic concentration limits. However, he must have been near the border line, for he states: "Die Schädigung durch Ozon war bei manchen Versuchen so gross, dass oft ganze Versuche abgebrochen werden mussten." His results show, as summarized also by KOERNICKE (**13**), that 35 control plants had a leaf surface of 741.37 sq. cm. while 35 treated plants had 1,431.32 sq. cm.; and 31 control plants had a dry weight of 19.057 gm. while 31 treated plants had a dry weight of 33.655 gm. Control plants transpired, during five days, 1299 gm. of water, and the treated plants 1554 gm. Also, during three weeks, when the plants were grown in a nutrient solution, the intake of salts was 7 mg. higher per treated plant than for the controls. Other experiments showed that when the plants were placed in a cellar, a three-peaked curve of leaf motion of small amplitude was obtained, which became a normal two-peaked one of larger amplitude again after "ionization." With stronger ionization, stronger movements were obtained, and with strong ionization the leaves rose. He claims that plants in ionized air remained green in the dark for a longer period; and that when grown in "ionized" air they were darker green and often had a fourth leaflet. This latter observation was emphasized and photographs recorded. This emphasis seems unreasonable. It so happened in the writer's work that this fourth leaflet appeared frequently, but more often in the untreated plants than in the treated.

The above results alone are interesting, but the following criticisms make them untenable:

(1) At all times LIPPERHEIDE used both ionized air and ozone, with possibly small amounts of nitrogen oxides, the amount of O_3 produced depending on humidity, potential used, etc. Up to the time his work was done there had been nothing to show that O_3 in small dosages might not be beneficial. Therefore, assuming all other conditions constant, one would not know to which to attribute the effect. One could possibly assume that when the concentration of O_3 passed a certain point the plants were harmed. This

evidently often occurred. Also, he gives no data on point distances, current, or potential used.

(2) Evidently there was no method of controlling air flow. It seems that all that was done to the control case was to open the door while he was blowing the air past the discharge into the treated chamber, "Dadurch wurde es vermieden, dass den Versuchpflanzen mehr frische Luft zugeführt als den Kontrollen."

(3) How light was controlled is not discussed. It seems it would be very difficult to place two cases in a greenhouse so that the light penetrating the cases was at all times equal.

(4) Where soil was used, no mention is made of how soil moisture was controlled.

(5) Definite mention, however, is made that the humidity varied considerably at times when the readings given were made, there being variations of 25 to 30 per cent. Also some differences are given for temperature. The blower connected with the treated case was evidently only in operation when a discharge was going on between the points. During the remainder of the time, the door of the control case was opened an amount supposed to approximately equalize the effect of the ventilator of the treated case. There is nothing in the description of the construction and setup of the cases which would show that a significant difference in humidity might not have existed most of the time. The writer's work with the same plants indicates that *Phaseolus vulgaris* is particularly susceptible in its growth and size of leaves to moisture conditions.

(6) The work may be criticized because of the small number of plants for which data are given; also, it is not clear on what basis the plants thought to be injured by ozone were eliminated.

(7) From the diagram shown in his article, it appears that the ultraviolet light from the sparks might reach the plants, perhaps having some effect.

In all other experiments listed in the bibliography, no measurements were made, as for instance in the numerous experiments of NEHRU (**17**) in which a variety of sources of supposed electrical energizing were used and seemed in almost all instances to produce stimulating effects.

Lack of controlled conditions as to light, temperature, air flow, etc., are general, as is also the use of a limited number of plants, or the taking of readings of respiration over very short periods, or other similar procedures which would tend to emphasize idiosyncrasies of individuals or of small groups, or differences due to time of day and other local factors. An example of this is the case in which NEHRU (**17**) after uprooting 5 plants, treated them with an electric spark and used only one plant for a control.

Then there exists in some cases questionable accuracy of measurements by absorption or colorimetric methods of the small amounts of CO_2 involved in small differences in respiration.

Available information on effects of ozone on plants

The earliest mention of studies of the effects of ozone on plants was found in a report by HABER (5) of the Kaiser Wilhelm Institute who presented his results at a special meeting. This work has remained unpublished, but the following quotation is taken from the above report:

"He gave the results of his investigations with others of the assimilation of a leaf of cherry laurel in air filled with carbonic acid. It was found that the electric field, either continuous or alternating, produced no change in assimilation unless a glow discharge was obtained. The reaction produced by the glow discharge injured assimilation. The admixture of ozone or oxide of nitrogen with air had the same effect."

The information that this work remained unpublished is taken from STERN (24).

KNIGHT and PRIESTLY (11) passed air through a jar in which an electrical discharge was taking place and thence into a jar containing seedlings. They found the chlorophyll in the upper portions of the seedlings had been bleached. They made no measurements of the products of the discharge but concluded there was no effect on respiration. The bleaching, as will be shown later, was, no doubt, due to a fairly high concentration of ozone.

LIPPERHEIDE (14), in a short paragraph on the influence of ozone on leaf movements of *Phaseolus,* states that the leaves go into the "sleep position" when subjected to a moderate amount of ozone. He finds that the leaves are very sensitive to ozone. If ozone was present for over five minutes, the plants were injured, and after two days were speckled brown, and parts were dead. He adds that ultraviolet rays also cause a downward movement of the leaves and attributes this to ozone arising from the action of ultraviolet radiations on air. No analysis of the gas used was made.

According to authoritative opinion (9), ozone in sufficient concentrations to kill bacteria will kill animals. Apparently, beneficial effects of ozone are due to its odor, which gives relief to the olfactory sense from the tedium resulting from constant inhalation of air at constant humidity and temperature. Also, beneficial effects in diseases of the mucous membranes, if any, are the result of blistering action which may cause increased blood supply.

TAKAMIYA (25), in repeated experiments, has shown that ergosterol exposed to ozone in proper concentration gives a substance having the action of vitamin D, as it also does in ultraviolet light. Thus there might be some beneficial reaction with plant sterols, although work reported in this paper does not indicate it.

Ozone is not found to any extent in lower altitudes due to its decomposition by moisture. In the upper stratosphere, measurements have shown (**15**) that there is an equivalent of a layer about 4.2 cm. thick. MELLOR (**15**) emphasizes the importance of this by quoting B. MOORE as follows:

"As the light from the sun first strikes the earth's atmosphere it is full of ultra-violet light and violet light. Such light rays as these, if they reached the earth at the present moment, would annihilate us; they would simply mean death to the world below. The first thing that happens to prevent this is that ozone is developed, and by absorption this ultra-violet light is shut out."

In regard to the possible production of ozone by plants, URSPRUNG and GOEKEL (**27**) obtained an effect on a photographic plate placed in a closed chamber with plants. As their electrometer showed no change, they concluded that the effect on the plate was due to ozone or other gaseous products and not to ultraviolet radiations which would ionize the air. Results that seem to confirm this idea were given by COUPIN (**3**). A concentrated solution of KI in litmus paper was placed in a chamber with plants and also in a control chamber, and exposure made to diffuse light. The litmus turned blue only in the plant chamber. Evidently some oxidizing agent, perhaps ozone, was formed by the plants which liberated KOH.

Plan of experiments

From the summary just presented, the writer concludes that little can be definitely said, except that O_3 at certain high concentrations was undoubtedly toxic. The possibility remained that ionization alone might have been stimulating, or that ozone in proper concentration might be beneficial to growth. It was, therefore, decided to carry out the following plan:

(1) Determine a method of growing a number of plants at a time under exactly controlled conditions so that it would be possible to change only the gaseous environment of the plants.

(2) Find the best method of producing a high concentration of gaseous ions uncontaminated by ozone.

(3) Devise a method of producing ozone in various desired concentrations without nitric acid anhydride (N_2O_5) being present in any detectable amount.

(4) Grow plants (*Phaseolus vulgaris*) for a sufficient length of time (seedling to blossoming) so that any effect on growth would become definitely evident.

(5) Grow plants in different concentrations of ozone, to determine what concentration is toxic, the specific effects produced, and whether different small concentrations applied either constantly or intermittently are stimulating.

Apparatus and methods

Growth chambers

The plants were grown in experimental cases of the design and dimensions illustrated in figures 1 and 2. The top is set in a trough completely surrounding the sand pan and may be lifted off. This trough is filled with water or other suitable liquid, which acts as an air seal. The glass sides leave the plants visible, while the easy removal of the glass case from over the plants makes it convenient to get at them. To assure complete drainage, the bottom of the sand pan was slightly funnelled.

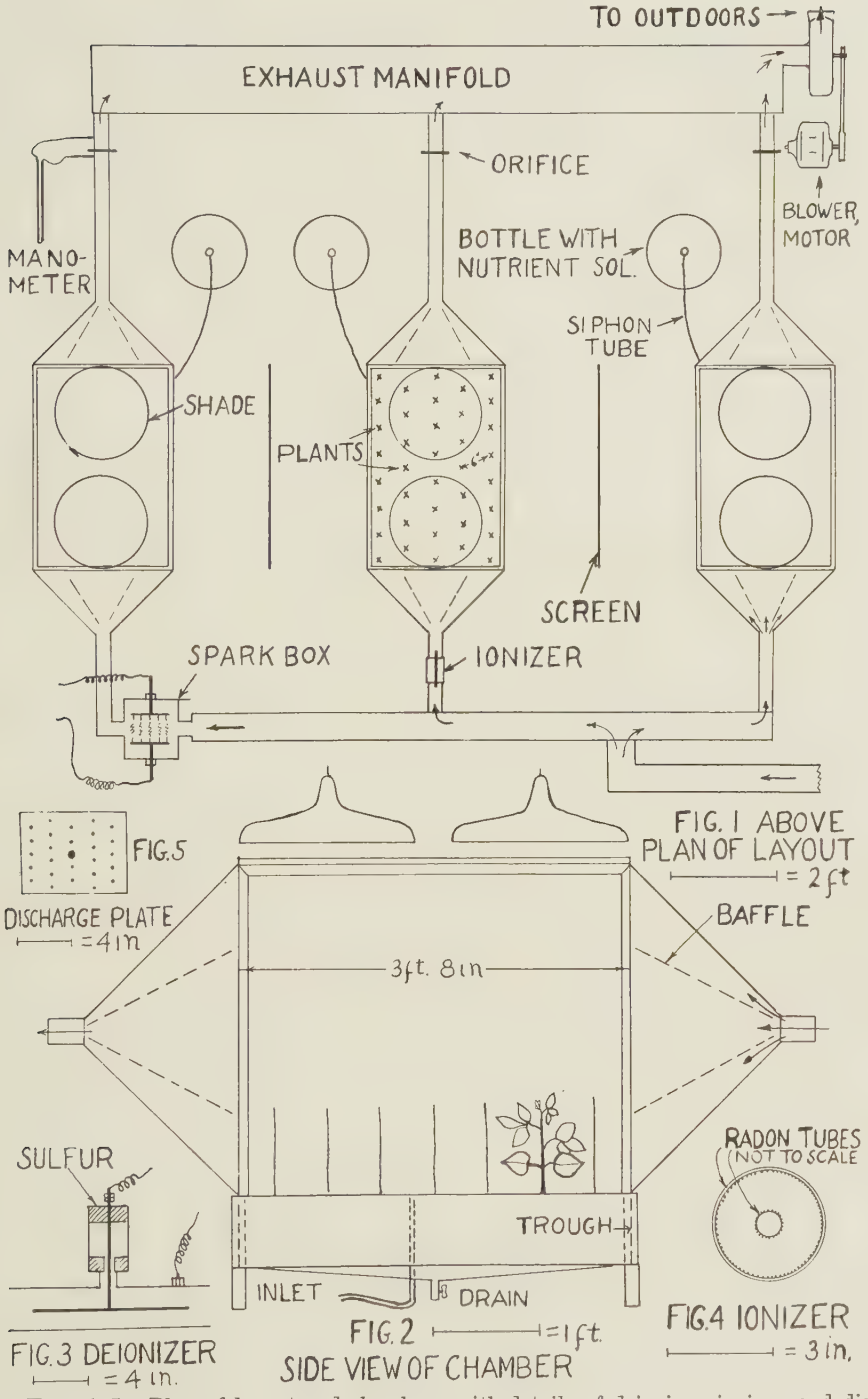
Baffles are soldered into the funnel ends so that air entering and leaving the case is spread out evenly through the interior and does not pass only through the center region. The baffles and funnels are painted dull black to avoid reflection of light. Around the top of the case is fastened a band so that the top glass may be covered with a layer of water.

Three cases were placed in parallel between a common outlet and inlet. One case served as a control for the other two, thus reducing by half the time which would be needed if only two cases were used. Air was drawn through the cases by an exhaust centrifugal blower. Lighting was accomplished by placing incandescent bulbs with reflectors over the cases. The gaseous products were introduced at the inlets.

Control of air flow

In order to be able to maintain equal air conditions in the cases, it was imperative that the air flow be accurately measured and regulated. The common exhaust manifold was 15 inches in diameter so that there would be no drop in pressure with distance from the outlet.

Measuring and regulating air flow was accomplished by the method of differential pressure across an orifice plate placed in the line. The principle is based on Bernoulli's theorem (**12**). If a pipe of perfectly true bore is used, and an orifice plate inserted so that a perfectly round hole is accurately centered, and taps then taken at proper distances, the flow through that pipe may be known with an accuracy of 0.5 per cent. Tables have been worked out (**22**), so that, knowing the fluid and its density, the inside pipe diameter, the ratio of the orifice to diameter of the pipe, and the differential pressure across the orifice, the flow may be found. Correction factors, (also obtained from tables) are introduced for temperature, pressure, and humidity. The tables used here were kindly furnished by the Republic Flow Meters Company of Chicago, Illinois. Of course, the accuracy of the determination of the flow depends entirely on the accuracy with which the diameter of pipe and orifice, and the differential pressure reading are obtained. Seamless steel tubing was used which varied only 1 to 2 thousandths of an inch, and sufficient length was used in front of the orifice so that any turbu-



FIGS. 1–5. Plan of layout and chambers, with details of deionizer, ionizer, and discharge plate.

lence in the flow would be smoothed out. The flange, fitting over the pipes and holding the orifice plates, and the orifice plates themselves, fitted together with the orifice exactly centered. The size of the orifice and the speed of the blower were adjusted by trial so that the flow desired could be obtained with a differential pressure of 70–100 mm. of water, which differences could be read within a fraction of 1 per cent. Even greater accuracy than 0.5 per cent. could be obtatined when simply balancing the flow through two or more identical pipes and orifices, and in reading the differential pressure for accurate adjustment. In this work the limit of error was not over 1 per cent. The greatest errors come from inaccuracies in knowing the diameters of the pipe and orifice, which are perpetuated as the square, while those in reading the differential pressure vary only as the square root.

The blower used was of the ordinary centrifugal fan type (Buffalo Forge Co. no. 1) and was driven by a synchronous motor to avoid changes in flow resulting from fluctuations in line voltage, which was found to vary as much as 10 per cent. when an induction motor was used. As the pressure on the exhaust side of all three orifices was the same, the flow was balanced through the cases by simply adjusting the inlets so that the differential pressure across the orifices were identical. Since the sizes of the three cases and pipes leading in and out of them were all the same, very little adjustment was necessary. It was found that a flow of about 30 cu. ft./min. was suitable, as this changed the air about once a minute in the cases, caused only very trifling movement of the leaves, and when ionized air was being used, was sufficiently rapid so that too large a degree of recombination did not take place. Since air entered the cases from a common manifold and passed through rather rapidly, it was not to be expected that the humidity would differ in the different cases. Measurements of the relative humidity in the three cases showed that there was no detectable variation.

Control of light and temperature

Over each case, at a distance of two inches from the glass, were suspended two 1000-watt bulbs with reflectors. These bulbs were tested for equality of illumination. The glass of the tops was $\frac{1}{8}$ in. thick, the sides $\frac{3}{16}$ in. and of a special high-grade crystal type of glass which was uniform in light transmission and thickness. The top glasses were covered with $\frac{3}{8}$ in. of distilled water, maintained at constant level by an overflow and drip source of replenishment. As dust settled to a slight extent in spite of the fact that the experiment was carried on in an air-quiet basement room and that the reflectors largely covered the tops, the glasses were cleaned daily. Without the water on top, and with an air flow of 30 cu. ft./min., the temperature of air leaving the chamber was 2° or 3° C. higher than that entering; while with the water screen, it was only a fraction of a degree higher. The temperature

in all three cases was equal. But the water was used throughout, not particularly to lower the actual temperature of the air in the cases but to eliminate a large proportion of the infrared rays and so to give a better balanced spectrum and also to prevent internal heating of the leaves. It was found that the plants used showed no curvatures in any direction and grew to uniform heights. There was, therefore, little or no error introduced due to one set of plants growing nearer to the lights, which might tend to exaggerate the differences in growth. Light screens were placed opposite the glass sides of the cases to prevent light from one case being reflected into another.

Control of soil conditions

The plants were grown in pure quartz sand, eight inches deep. Constant soil moisture conditions were obtained by flooding once a day with nutrient solution and draining immediately after. This was accomplished by placing a five-gallon bottle of nutrient solution somewhat above the pans and allowing the solution to siphon to the surface of the sand through a $\frac{1}{2}$ in. pipe from the bottom where it poured into a $1\frac{1}{2}$ in. angle iron with holes bored along its sides. In this way, the liquid flowed fairly uniformly throughout the length of the pan into the sand without disturbing it. When the sand was immersed about $\frac{1}{4}$ in. in the liquid, the inflow was shut off and the drain opened. As the sand in all these pans was of the same particle size and of the same packing tendency, the amount of solution held should have been pratically identical in all three cases. That this was true was shown by measurements of the amount entering, and of the excess that could be drained. Thus soil moisture conditions were constant at the start and would be changed only by differences in degree of transpiration and evaporation during the twenty-four-hour period between waterings. However, there was ample moisture in the sand to last several days and tests showed that it took practically the same amount of water to saturate the sand in each of the three pans after twenty-four hours of transpiration and evaporation. Fresh air was also sucked into the sand when the liquid, filling the intergranular space, was drained.

The nutrient solution used was as follows:

A.	50 gm.	$MgSO_4$	in	500	cc.	H_2O
B.	50 "	KH_2PO_4	"	"	"	"
C.	50 "	$Ca(NO_3)_2$	"	"	"	"
	50 "	$CaCl_2$				
D.	4 "	$FeCl_3 \cdot 6H_2O$	"	300	"	"

Add 10 cc. of solutions A, B, and C, and 10 drops of D to four liters of distilled water.

Obtaining plants of similar size and vigor

About 1000 *Phaseolus vulgaris* seeds of uniform size were selected each time and planted in quartz sand. They were found to germinate uniformly and from this number of plants it was not difficult to obtain 99 plants of almost identical appearance at an early stage in the development of the primary leaves (about ten days after planting). Thirty-three were then planted in each pan (fig. 1), six inches apart and four inches from the sides, in the case of the two rows nearest the glass. At this distance there was little crowding and the plants were not interfered with nor shaded by one another. The plants grew very uniformly and no differences could be noted between the outside and inside rows.

Production and measurement of ionized air

Three sources of ionization of the air drawn into the cases were considered: spark discharges, radioactive compounds, and x-rays. It was at first thought that spark discharges would serve best for a constant source of quantity production of ions and since Lipperheide used this method, it was tried first. A spark discharge box (fig. 5) was built. It consisted of two plates each having twenty-five points spaced 1 in. apart. These were capable of being adjusted so that the points of the opposite plates could be placed at any distance from one another up to 8 in. The two plates were connected to the terminals of the secondary of a transformer that could be regulated up to 100,000 volts. When the current was turned on, a lively display of sparks played between the points. Large Leyden jars (3000 m.m.f. capacity) were placed in parallel with the plates to prevent arching. In this way blue-white, crackling, thick, hot sparks were obtained, which type forms the least ozone. Air was then drawn between the points through the sparks into the plant chambers.

Another method of producing spark discharges was to use a Tesla coil discharge (frequency 4,500,000 cycles per second) connecting the two terminals of the secondary connected to the plates. This gave thinner purplish sparks that became a spray of fine thin sparks spreading between opposite points. This changed almost to the appearance of a glow discharge filling the space between the plates when the points were moved farther apart (6 to 8 in.). The thin sparks produced by the Tesla coil when the points were drawn 4 to 6 in. apart spread out from the points so that practically all the air between the points had a visible discharge in it.

In order to measure the number of ions per cc. in the air entering and leaving the plant chambers it was necessary to build a device which could be inserted in the air line and by which all of the ions could be discharged. As these were passing in at a constant rate, a constant current would result that could be measured on a galvanometer or electrometer. A Dolzalek

electrometer was used (capacity 93 e.s.u.) because, by inserting different capacities in parallel, it could be made flexible in use over the range of currents to be measured and because a galvanometer that was sufficiently sensitive for some of the small currents to be measured was not available.

The device through which the ionized air passed into the chamber is shown in figure 3. It consisted of a brass tube of the same size as the pipe entering the chamber. A brass rod was suspended longitudinally down the center of the tube from its middle by another rod passing up through two cakes of sulphur held by a glass tube with its lower end drawn in but not touching the brass rod, so that the surface of the sulphur would not become contaminated from dust, moisture, etc., in the air passing through the tube. The use of sulphur was found necessary. At first, glass alone was used, and there was a considerable leakage over 12 inches of glass tubing from which the rod was suspended at the top, due to surface moisture. The outside tube, which was completely insulated from connecting pipes, was charged up to the proper voltage by connection to one pole of a battery, the other of which was grounded.

A lead was taken off the suspended rod to one quadrant. Because of the strong electric waves given off by the spark discharges, which would greatly affect the electrometer, it was completely shielded in a galvanized iron box, the beam of light for reading passing through a screen. The lead from the suspended rod and the part projecting up above the brass tube was shielded by metal tubing. It was not found necessary to place screens across the ends of the brass tube because the suspended rod, placed only within 2 inches of each end, was found to be so completely shielded as not to affect the electrometer. Then, by first placing the quadrant with suspended rod and lead at ground potential and with the outer tube charged, all ions of similar charge were driven to the brass rod where they gradually charged the quadrant up to the potential of the outer tube. The rate at which the quadrant charged up, and thus the rate of turning of the needle as measured on the scale, depended on the number of ions available to carry the current across from the outside tubes to the rod, and also on the capacity of the quadrant, lead, and rod. Knowing the capacity of the system (found by the usual method of calibrating with a known capacity), the charge of a single ion, the number of centimeters of scale corresponding to a change of 1 volt in the potential of the quadrant, and the rate of flow of air in cubic feet per minute, the number of ions per cc. may be calculated as follows:

$$2 \times \frac{\dfrac{(Ce + Cc)\ (V/cm.) \times (\text{number of cm.})}{t}}{(4.77 \times 10^{-10}\ \text{e.s.u.})\ (30.48\ \text{cm.})^3 \times \dfrac{28.6\ \text{cu. ft. per min.}}{60}} = \text{no. of ions per cc.}$$

Explanation of symbols:

Ce = capacity of electrometer and leads (93 e.s.u.)

Cc = capacity of air condenser inserted in parallel

V/cm. = number of volts to which 1 cm. corresponds on scale placed at one meter = 1.2

Number of cm. = number of cm. taken for reading

t = time in seconds for the needle to move the above number of cm.

4.77×10^{-10} e.s.u. = one elementary unit of charge, *i.e.*, charge on one ion

$(30.48)^3$ = conversion factor for changing cubic feet to cubic centimeters

28.6 cu. ft. per min. = rate of flow of air per minute, divided by 60 to change to seconds

2 = factor for total number of ions as only one-half of the ions (as measurements showed there were equal numbers of + and − ions) are driven to the quadrant of the electrometer, the other half being neutralized by the charge of the tube.

It was found that charging the outside tube to 135 volts was sufficient to take out all of the ions at the rate of air flow used. Further increase in voltage gave no greater rate of movement of the needle. In fact, 90 volts gave, within a small percentage, the same reading as 135 volts.

In calibrating the electrometer several radon tubes were used as a constant source of ionization. These were suspended in the brass tube. It was found that the needle swung extremely rapidly and, in order to get the motion down so that readings could be made accurately, almost all of the tubes had to be removed. It was subsequently found that by placing the radon tubes at the entrance to the plant chamber (air flowing at the time) and the ionization chamber at the outlet, a higher reading was obtained than if the air had passed through sparks. In order to see how many ions per cc. could be produced with a flow of 28.6 cu. ft./min., a glass tube was coated on the inside with several hundred old radon tubes and another smaller glass tube suspended longitudinally in the larger tube, it being coated on the outside with radon tubes (fig. 4).

It was thus found that 1,100,000,000 ions per cc. could be produced at the near end with a flow of 28.6 cu. ft./min. That this measurement was correct was shown by placing three feet of pipe between the ionization chamber and the radon tubes. No current could be detected by the electrometer unless air was flowing through the pipe, showing that what was being measured was actually the number of ions per cc. and not the rate of production of ions by rays of some sort. At this point adding additional radon tubes did not increase the ionization perceptibly.

At the outlet of the chamber, owing to recombination of ions while passing through, the reading dropped so that only 21,000,000 ions per cc. were measured, but this is still a large number.

It was found that the Tesla coil discharge would give only about one-fifth of this number, and the maximum white spark discharge obtainable gave only about one-twelfth as many. This latter degree of ionization was obtained at a point distance of about 4.5 cm., when there was probably the greatest density of sparks and greatest potential drop per centimeter. Practically no ionization took place unless there was a spark or corona discharge. Sparking, and not the field alone, is evidently necessary to get a large degree of ionization by collision. This would indicate that in experiments with overhead wires little ionization takes place. The ionization readings remained constant and the maximum degree of ionization was used throughout the experiments using ionized air. Strangely enough, a spark discharge used as an auxiliary source with the radon tubes, decreased rather than increased the readings.

Owing to the fact that elaborate equipment would be needed to employ x-rays and eliminate errors and danger from their use, and that the tubes would not be constant sources, and could not be run for long periods, they were not tried.

Measurement and production of ozone

The air from the plant chamber was drawn through two Fisher wash bottles containing KOH solution and through two containing neutral KI solution at a constant known rate. This rate was determined by calibration of a capillary flow-meter and kept constant by regulation of the differential pressure. It was found, in calibrating, that if the differential pressure was set at a certain point, the time of flow of a certain volume of air could be predicted within a fraction of 1 per cent. The rate used was about one-half liter per minute. The iodine liberated according to the equation

$$2KI + O_3 + H_2O = 2KOH + I_2 + O_2$$

was titrated in acid solution according to the method described by Treadwell and Hall (**26**).

$$I_2 + 2Na_2S_2O_3 = 2NaI + Na_2S_2O_6$$

or 2 moles of $Na_2S_2O_3 = 1$ mole O_3. Thus knowing the volume of gas drawn through the absorption bottles and the number of cc. of 0.01 molar $Na_2S_2O_3$ solution used, the number of parts of O_3 is easily determined.

$$\frac{\frac{1}{2}\text{ number cc. of 0.01 M } Na_2S_2O_3 \times \frac{22{,}400}{1000 \times 100}}{\text{(Number of minutes of absorption) (cc. per min.)}} = \text{Parts of ozone per one part of air.}$$

(Corrected for standard conditions)

To produce O_3, and to be able to regulate the amount, a Siemens type of ozonizer was built (diameter 4 in.). Regulation of the concentration of ozone could be accomplished by changing the area of the outer layer of foil, by changing the potential, by changing the cross sectional open areas between the glass tubes or the amount of open area of the inside tube, thus causing more or less air to flow relatively between the tubes where it was ozonized by the strong alternating electric field. Twenty to thirty thousand volts could be used without sparking. The air, after being ozonized, was passed through the deionizer.

It was soon found that only small concentrations of O_3 were necessary and at none of the concentrations used was any N_2O_5 detectable by passing large volumes of the air through KOH and testing the resultant solution by several of the most sensitive qualitative tests for NO_3. All lower oxides that may be formed are immediately oxidized to N_2O_5 by O_3 (**4**).

Experimentation

Experiments 1 and 2

Thirty-three seedlings were transplanted into each chamber. The primary leaves were about one inch across. Lighting was for 16 hours daily. Ionization was at a maximum rate and was continuous, using the radon tube device. The sand was saturated and drained every twenty-four hours.

The plants grew rapidly and bloomed nineteen days after transplanting, and twenty-nine days after planting of the dry seeds in moist sand. Especially noteworthy was the uniform growth of plants and uniformity of time of blossoming in the two chambers. The leaves were unusually large and the internodes elongated, but there was practically no lodging. The plants were all about twenty-three inches high. The treated and control plants were identical in appearance. Also, there were no differences to be observed between the plants growing near the inlet and those near the outlet.

The chambers were alternated between the control and treated sets in the two experiments so that if there were any differences due to conditions in the chambers they would be discovered. There was, as shown, a large increase in dry weight, indicating that the plants grew considerably, and any effect due to the treatment should have been noticeable. As indicated in the last line of table I, the treated plants weighed 1.7 per cent. more in experiment 1, and 2.2 per cent. less in experiment 2 than the controls. There was thus no significant difference apparent from growing in highly ionized air.

Experiments 3 and 4

Since the previous experiments show no differences by using bright light, and as Henrici's results indicated that the effect on assimilation was more pronounced in dim light, it was decided to grow two sets as in experiments

TABLE I

RESULTS OF EXPOSING BEAN PLANTS TO IONIZED AIR
DATA FOR THIRTY-THREE PLANTS

	Experiment 1		Experiment 2	
	Treated	Control	Treated	Control
	gm.	*gm.*	*gm.*	*gm.*
Fresh weight, tops	406	393	375	387
Fresh weight, roots	88	95	77	82
Dry weight, tops	49.1	47.9	45	46.1
Dry weight, roots	6.8	7.1	5.2	5.3
Total dry weight	55.9	55.0	50.2	51.4
Weight of 33 seeds	10.94			
Weight of 33 seedlings, dry	7.3			
Percentage increase during period of treatment	766%	753%	688%	700%
Percentage difference of treated	+1.7%		−2.2%	

1 and 2, except that the bright lights (1000 watts) were on for only eight hours and were replaced with 100-watt bulbs for the remaining sixteen hours of each day. Under this changed condition, the plants did not grow as well. They were shorter, the leaves were smaller and seemed less succulent. A large percentage of the blossom buds aborted, the immature blossoms falling off before maturing. There were no apparent differences between the treated plants and controls. The dry weight of thirty-three plants was much less than before (table II). The figures in the table indicate how uniform the conditions of growth must have been.

Experiment 5

Again using ionized air two hours in the morning (9 to 11 A. M.) and two hours in the afternoon (3 to 5 P. M.), and lighting each case with two 1000-watt bulbs, as in experiments 1 and 2, no significant effect was obtained (table II).

TABLE II

RESULTS USING LIGHT OF LOW INTENSITY AND IONIZATION INTERMITTENTLY
DRY WEIGHTS FOR 33 PLANTS

Experiment	Treated	Control	Percentage difference
	gm.	*gm.*	%
3	34.0	33.4	+1.8
4	31.0	31.0	0.0
5	47.5	48.7	−2.5

In the above experiments with ionized air the radon tubes, rather than a spark discharge, were used because the high frequency could not be cut down to a point where the O_3 produced did not kill the plants. Using the blue-white sparks direct from the secondary, with condensers in parallel, it was found that also here, unless the sparking and potential were cut down to a point where relatively very few ions were produced, the ozone was still excessive. Radon produced no detectable O_3.

Experiment with ozone

In this experiment the procedure was to start with applications of relatively high concentrations of ozone, 1 part in 50,000 to 100,000 of air, in which case results were visible in a few minutes, and then decrease the concentration until either no toxic or no beneficial effects were indicated. Bean plants were used as before.

It was found, first, that concentrations of one part of ozone in 100,000 of air resulted in a wilting of the leaves in 15 to 30 minutes. The lower and older leaves succumbed first, while the very youngest were not affected. The ozonizer was then disconnected and the plants allowed to recover, if still capable of doing so. The wilted leaves did not recover but became bleached, giving a yellow enameled appearance. In those less affected there remained green stripes along the larger veins, while in others the part of the leaf nearest the stalk appeared unaffected.

The topmost younger and not fully expanded leaves were unaffected. The plants were allowed to continue to grow, but, although they bloomed at the same time as the controls, they were stunted and their dry weight, including the bleached leaves, was only three-fifths as much.

With concentrations of one part of ozone in 1,000,000 of air applied only for periods of 30 minutes twice daily, the plants presented a somewhat stunted and dried up appearance, and in eight days brown spots appeared on the older leaves. If this concentration was applied continuously, the plants showed a slightly withered appearance of the leaves and brown spots appeared on the second day.

With a concentration of 1 part in 3,000,000 of air there was no noticeable desiccated appearance of the leaves, and the spotting occurred in four days, always on the older leaves. If the application was continued after this, the spots increased in size and the leaves dried up and fell off, the younger leaves gradually being affected, and then the stem.

With concentrations of 1 part in 6,000,000 of air the spotting did not occur until the twelfth day, the plants appearing normal until then.

With a concentration of 1 part of ozone in 8,000,000 of air, a slight speckling was shown in three weeks.

Another set of plants was grown at a concentration of 1 part of ozone in 10,000,000 of air, applied during two one and one-half-hour periods daily,

and another set at a concentration of one part in 17,000,000 of air continuously for eighteen days. In neither case was there any noticeable change in appearance nor significant difference in the dry weights which were 41.8 and 43.4 gm. for the two treated lots respectively, and 42.9 gm. for the control.

In the above cases of spotting, closer examination showed that the spots originated mostly in the islets between the smaller veins, where there was also a greater density of stomata. The affected cells appeared darkened, desiccated, and collapsed. Similar effects at like concentrations were found for such plants as *Abutilon* and *Coleus*.

Since it was noted that ozone in sufficient concentration had a bleaching action on chlorophyll and it seemed reasonable to assume that such a strong oxidizing agent might accelerate respiratory or aging processes, it was decided to try the effect of different concentrations of ozone on the rate of ripening of green bananas and tomatoes. Results indicated that no change occurred in the rate of ripening of bananas or tomatoes. The conditions were such that the fruit ripened excellently, but unless sufficient concentrations of ozone were used to produce brown spots, no effect was noted. Much higher concentrations of ozone (1 part in 30,000) were required to spot the fruits than was found to so affect the leaves, probably due to the lack of stomata and greater cutinization of the epidermis of the fruits.

The Tesla coil and discharge box being available, it was thought advisable to determine what effect, if any, the Tesla discharge might have on dry and germinated seeds. Consequently dry seeds and germinated seedlings of corn and sunflower were treated for periods of various lengths. The seeds and seedlings were suspended on a heavy sheet of paper between the discharge points of the two plates. Only the fine spray sparks were used and not the cracking ones, also various point distances were used. They were thus subjected to an intense high frequency field and a high concentration of ozone. No effect was noted on the growth of the plants unless the seeds were exposed for as long as 12 minutes at a point distance of 4 cm. and frequency of 4,500,000 cycles per second. This treatment resulted in stunted plants, most of which did not continue to grow after eight to ten days, although none of the seeds were killed outright by the treatment.

Discussion

The writer thus finds that the results of KOERNICKE, and of LIPPERHEIDE to the effect that ionization of the air promotes growth are not confirmed. The writer used a much higher degree of ionization, both continuously and intermittently, than did LIPPERHEIDE; also, the degree of ionization varied greatly between the entrance and exit, and no effect of ionization gradient was noted. It is not likely that such low degrees of ionization as LIPPER-

HEIDE must have used could have had any effect on growth. His lack of descriptive information makes it impossible to duplicate his conditions. We cannot use increased ionization of the air, therefore, to explain some of the previous results of electroculture but must fall back on criticism of control of conditions.

In regard to ozone, the necrosis of only local areas at first, and these on the older leaves, is difficult to explain. Usually with toxic gases the younger parts are first attacked. Microscopic examination showed that the spotting occurs in areas where the stomata are more numerous. It is suggested that the action of ozone is partly the saturation of lipoid compounds in the cell membranes, resulting in loss of semipermeability, wilting, oxidation of cell contents, and death of the cell.

Since it is difficult to summarize completely the errors in previous work that indicate opposite results to those described in this paper without too much repetition, the reader is referred to the section on results of previous investigators. It may be said here, however, that major mistakes resulted from not isolating the factors, not measuring the conditions, measuring rates of production of ions rather than concentration (actual number per cc.), not controlling other conditions, such as humidity, light, etc., not using a sufficient number of plants, and the use of very weak sources of ionization. The reader is requested to check the methods developed in this paper to assure himself that the technic here used did not admit of any conditions that would invalidate the results.

It may be said that in the experiments herein described plants for the first time have been grown for a period in conditions where the factors of ionization of the air, and ozone, were isolated. The writer contends that examination of growth over a period (from seedling to blossoming) is more likely to show the effects of these factors than measurements of assimilation or respiration over short, intermittent periods.

Summary

1. Plants grown in highly ionized air (1,100,000,000 ions to 21,000,000 per cc.) showed no significant changes in appearance or dry weight over controls, whether the treatment was applied continuously or intermittently with bright or with dim illumination.

2. Ozone is toxic and produces necrotic spots with final desiccation of the leaf in concentration of 1 part in 1,000,000 to 1 in 8,000,000 of air.

3. Lower concentrations of ozone, which are still easily detectable by odor, produce no stimulation of growth or other effects on the appearance of the plants.

4. Higher concentrations, about 1 part in 100,000, produce prompt wilting and bleaching, the older leaves succumbing long before the younger upper ones.

5. Ripening processes in tomato and banana are not accelerated by O_3. High concentrations produce spotting. These are much higher than concentrations required for spotting of leaves.

6. A Tesla discharge had no effect on dry or germinated seeds unless exposure was for twelve minutes, when stunted plants that did not develop were produced.

7. A method is developed that would be applicable to a study of the effects of different gases on plants under controlled conditions.

UNIVERSITY OF CHICAGO

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